Post-Prandial Compositional Changes of Fluid- and Particle-Associated Ruminal Microorganisms

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POST-PRANDIAL COMPOSITIONAL CHANGES OF FLUID- AND PARTICLE-ASSOCIATED RUMINAL MICROORGANISMS[1]

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ABSTRACT

Two ruminally cannulated cows were fed a diet containing 65% alfalfa haylage and 35% concentrate every 12 h. During two collection periods, whole ruminal contents were sampled before feeding, when feed not eaten was removed (1 h after initiation of feeding) and 1, 2, 3, 4, 6, 8, 10 and 12 h after removal. At each sampling, fluid-associated microorganisms were obtained by straining whole ruminal contents through eight layers of cheesecloth. A portion of the particle-associated population was obtained by chilling squeezed particles before seven successive extractions with saline solution. Microbial ash concentrations ranged from 9.9 to 16.6% of dry matter, with differences due to population ($P<.01$) and to time after feeding for both fluid- ($P<.05$) and particle-associated ($P<.01$) microorganisms. Within 1 h after initiation of feeding, N decreased ($P<.05$) from a pre-feeding concentration of 9.5% of organic matter to 7.7% for fluid-associated microorganisms, and from 9.2 to 6.7% for particle-associated microorganisms. Both populations did not return to pre-feeding concentrations until 6 h after feed removal. Nucleic acid concentrations decreased ($P<.05$) in both populations after feeding, and did not return to pre-feeding concentrations until 6 h for fluid-associated and 4 h for particle-associated microorganisms. Nucleic acid-to-N ratios were higher ($P<.01$) in the fluid- than particle-associated organisms. Decreases ($P<.01$) in lipid concentrations due to feeding were observed only in the particle-associated fraction. Mean lipid concentration of particle-associated microorganisms was 22.4% of organic matter compared with 24.2% for fluid-associated microorganisms. Polysaccharide concentrations increased after feeding and remained higher ($P<.05$) until 6 h after feed removal for both populations. Peak polysaccharide concentrations were obtained 1 h after feed removal, and were 20.3% of organic matter for fluid-associated and 33.6% for particle-associated microorganisms. Results are interpreted to indicate that when studying chemical composition of ruminal microorganisms, measurements should be made on both fluid- and particle-associated microorganisms.
(Key Words: Rumen Microorganisms, Nucleic Acids, Polysaccharides, Microbial Lipids.)

Introduction

The ruminal microbial population is complex and consists of bacteria, protozoa, fungi and yeast (Hungate, 1966; Bauchop, 1979). Changes that occur within this complex population due to feeding are not well understood. Some studies with mixed ruminal bacteria indicate that chemical composition can change soon after feeding (Smith, 1975; McAllan and Smith, 1977). Also, ruminal bacteria can replicate rapidly in response to nutrient influx (Bates et al., 1985). However, Leedle et al. (1982) found a decline in numbers of viable ruminal bacteria after feeding. The authors suggested that this decline may be due to a large proportion of ruminal bacteria becoming attached tenaciously to feed particles and not being dislodged by the typical procedure of blending before straining through cheesecloth.

Quantitative data indicate that microorganisms associated with the ruminal particulate fraction can constitute a large proportion of the ruminal microbial population (Forsberg and Lam, 1977; Merry and McAllan, 1983). Due to the difficulty of separating adherent microorganisms from plant residue, most of the qualitative and quantitative information pertaining to ruminal bacteria has been obtained by straining whole ruminal contents through

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cheesecloth. In many studies, whole ruminal contents have been blended before straining to remove a portion of the particle-associated bacteria. However, recent results indicate that blending alone will not remove a significant portion of particle-associated microorganisms (Dehority and Grubb, 1980; Leedle et al., 1982; Craig et al., 1984).

In a recent study (Craig et al., 1987), we fed cows a 65% alfalfa haylage diet and used ^{15}N to quantify the microorganisms associated with both particulate and fluid fractions during a 12-h period after feed was removed. It was observed that the particle-associated microorganisms represented two to four times more organic matter in ruminal contents than the fluid microorganisms. The goal of this study was to analyze the same microbial samples (Craig et al., 1987) for changes in nitrogen, nucleic acid, lipid and polysaccharide concentrations.

Materials and Methods

Two lactating Holstein cows equipped with ruminal cannulae were fed approximately every 12 h. Diet dry matter (DM) consisted of 65% alfalfa haylage (60% DM), 30% high moisture corn and 5% soybean meal. Feed was available for 1 h; any feed not eaten was removed after this time. There were two 13-h collection periods, 14 d apart. During each collection period, whole ruminal contents were sampled from each cow immediately before feeding, when feed not eaten was removed (1 h after initiation of feeding) and 1, 2, 3, 4, 6, 8, 10 and 12 h after feed removal. Immediately after feed was removed, cows were dosed with 2 g of ^{15}N ammonium sulfate to allow for quantitation of particle-associated microorganisms (Craig et al., 1987). Fluid microbes were separated in suspension by squeezing whole ruminal contents through eight layers of cheesecloth, plus washing the squeezed particles once with .85% w/v saline (equivalent to 20% of the total strained ruminal fluid obtained). The saline was added to the particles and contents were squeezed again. This single step was conducted to remove residual fluid microorganisms from particulate matter; the wash solution was added to the strained ruminal fluid. This process may have removed some of the particle-associated microorganisms. However, fluid population was defined as microorganisms obtained in SRF plus one particle wash.

Particle-associated microbes were detached by weighing approximately 100 g of squeezed particles (wet weight) into a vessel containing 600 ml of extraction solution (.85% w/v saline containing .5% w/v formaldehyde and .1% w/v Tween 80). After chilling (4 C) for approximately 24 h, contents were strained through eight layers of cheesecloth and the residue was washed seven successive times with .85% saline. Microbes (from both populations) were obtained from suspension by centrifugation at 30,000 × g for 30 min. Deposits were washed once with .85% w/v saline solution and recentrifuged. Samples were lyophilized for storage before analyses. Using ^{15}N as a microbial marker, 32 to 52% of particle-associated microorganisms were removed by the extraction procedure (Craig et al., 1987). The assumption is made that the fraction removed is representative of the total particle-associated pool. It is recognized that firmly-associated microorganisms may not be adequately represented in this study.

The samples, representing two microbial populations (fluid- and particle-associated) at 10 time-points with respect to feeding, were analyzed for four major groups of organic constituents. Polysaccharides (plus reducing sugars) were measured by the colorimetric phenolsulfuric acid method (Hellebust and Craigie, 1978) after 1 N sodium hydroxide digest of 20-mg samples, with glycogen as standard. Total lipids were determined using a modification of Hanson and Phillips (1981). Samples (200 mg) were extracted with a chloroform-methanol mixture (2:1, v/v) for 36 h in a Soxhlet extraction apparatus. After extraction, samples were dried at 100 C, weighed and the quantity of lipid material removed was determined. Nucleic acids (plus other purine derivatives; NA) were quantitated by measuring the ultraviolet absorption of purines, which were isolated from perchloric acid digests of 20-mg samples by precipitation as a silver complex (Zinn and Owens, 1982). Yeast ribonucleic acid was the standard. Nitrogen was determined by the micro-Kjeldahl method (AOAC, 1980). Total organic matter (OM) was determined gravimetrically by dry-ashing (AOAC, 1980).

Within each microbial population, transitory effects on percentage composition were tested by one-way analysis of variance (Steel and Torrie, 1980). Significance of differences between specific time-points within populations was tested with Fisher's protected LSD. Differences between populations across time-points

TABLE 1. ASH CONTENT OF FLUID- AND PARTICLE-ASSOCIATED MICROORGANISMS

Hours after feeding[a]	Population	
	Fluid-associated	Particle-associated
	% of dry matter	
BF	15.5^{def}	14.5^{de}
AF	13.7^{f}	10.8^{fg}
1	14.1^{ef}	9.9^{g}
2	13.6^{f}	10.8^{fg}
3	13.8^{ef}	11.9^{fg}
4	15.5^{def}	13.3^{ef}
6	16.5^{d}	13.5^{ef}
8	16.3^{d}	14.0^{de}
10	15.7^{de}	14.5^{de}
12	16.6^{d}	15.3^{d}
Overall mean[b]	15.1	12.8
SE[c]	.69	.62

[a]Samples were taken immediately before feeding (BF), 1 h after initiation of feeding (AF), when feed not eaten was removed and at intervals AF.

[b]Across all time-points, fluid microorganisms were higher (P<.01) than particle-associated microorganisms.

[c]Common standard error of means with n=4.

[d,e,f,g]Means within columns that do not have a common letter in their superscript differ (P<.05).

were tested with paired t-tests. Differences between populations at specific time-points were tested with individual t-tests.

Results

The ash content of both microbial populations ranged from 9.9 to 16.6% of dry matter (table 1). Differences in ash concentrations within population were observed over time for both fluid (P<.05) and particle-associated (P<.01) microorganisms. Also, the fluid microorganisms had higher (P<.01) ash concentrations than the particle-associated microorganisms over the sampling period. Due to temporal and population differences in ash concentrations, all constitutent comparisons are expressed on an OM basis.

Nitrogen concentrations decreased (P<.01) in both microbial populations within 1 h of initiation of feeding (figure 1). Nitrogen decreased from a before-feeding level of 9.5 ± .1 to 7.7 ± 3% in the fluid population and from 9.2 ± .02 to 6.7 ± .6% in the particle-associated population. In both populations, N did not return to before-feeding concentrations until 6 h after feed removal. A difference between populations was evident only at 1 h after feed removal, when the particle-associated microbes had a lower (P<.05) N concentration (7.5 vs 6.4%).

As with N concentrations, NA concentrations were lower (P<.01) than before-feeding concentrations within 1 h of initiation of feeding in both populations (figure 2). At 4 h after feed removal, particle-associated microorganisms had returned to before-feeding NA concentrations, while fluid microbial NA concentrations were lower (P<.01) than before-feeding concentrations until 6 h after feed removal. Fluid microorganisms had higher (P<.05) NA concentrations than particle-associated microorganisms

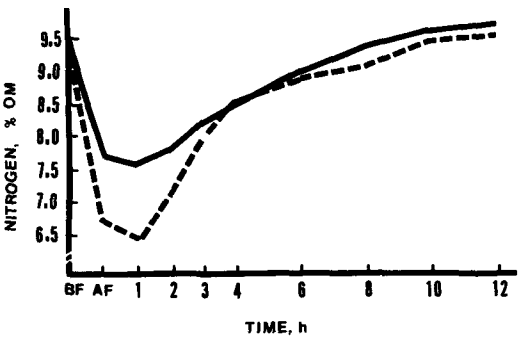

Figure 1. Change in nitrogen concentrations in organic matter (OM) of fluid- (▬) and particle-associated (▬ ▬) microorganisms. Standard error of treatment X hour means from analysis of variance was .13%.

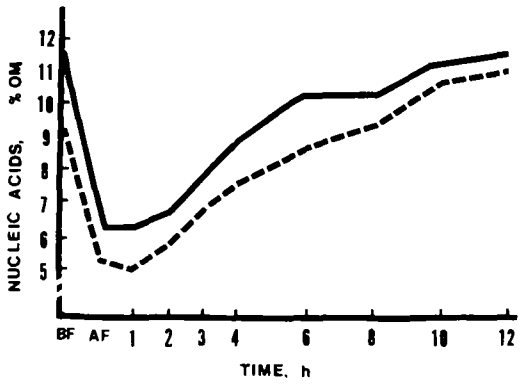

Figure 2. Change in nucleic acid concentrations in organic matter (OM) of fluid- (▬) and particle-associated (▬ ▬) microorganisms. Standard error of treatment X hour means from analysis of variance was .29%.

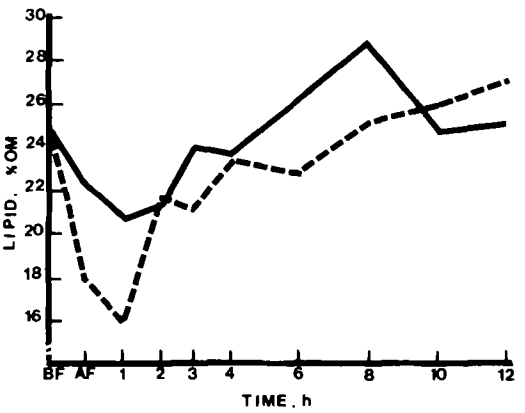

Figure 3. Change in lipid concentrations in organic matter (OM) of fluid- (▬) and particle-associated (▬ ▬) microorganisms. Standard error of treatment X hour means from analysis of variance was 1.1%.

across time-points. Nucleic acid concentrations of fluid microbes ranged from 11.5 ± .2% before feeding to 6.3 ± .5% 1 h after feed removal. The corresponding values at those time point for particle-associated microbes were 9.2 ± .1 and 5.0 ± .4%.

The ratio of NA to N also decreased (P<.01) in both populations soon after feeding (table 2). The decrease occurred within 1 h after initiation of feeding in both populations and remained different (P<.05) from before-feeding values until 4 h after feed removal, with the fluid microbes, and 2 h after feed removal with the particle-associated microbes.

A decrease (P<.01) in lipid concentrations after feeding was observed in the particle-associated microorganisms (figure 3). Lipid decreased from 24.2 ± .4% of OM before feeding to 16.0 ± .9% by 1 h after feed removal. One hour later it had returned to before-feeding concentra-

TABLE 2. CHANGES IN NUCLEIC ACID-TO-TOTAL NITROGEN RATIOS OF FLUID- AND PARTICLE-ASSOCIATED MICROORGANISMS

Hours after feeding[a]	Population	
	Fluid-associated	Particle-associated
BF	1.21^{d}	1.01^{de}
AF	.81^{g}	.76^{f}
1	.83^{g}	.78^{f}
2	.86^{g}	.78^{f}
3	.94^{fg}	.84^{ef}
4	1.05^{ef}	.87^{ef}
6	1.13^{de}	.96^{e}
8	1.07^{def}	1.01^{de}
10	1.16^{de}	1.12^{d}
12	1.18^{de}	1.15^{d}
Overall mean[b]	.98	.92
SE[c]	.05	.63

[a]Samples were taken immediately before feeding (BF), 1 h after initiation of feeding (AF), when feed not eaten was removed and at intervals AF.

[b]Across all time-points, fluid microorganisms were higher (P<.01) than particle-associated microorganisms.

[c]Common standard error of means with n=4.

[d,e,f,g]Means within columns that do not have a common letter in their superscript differ (P<.05).

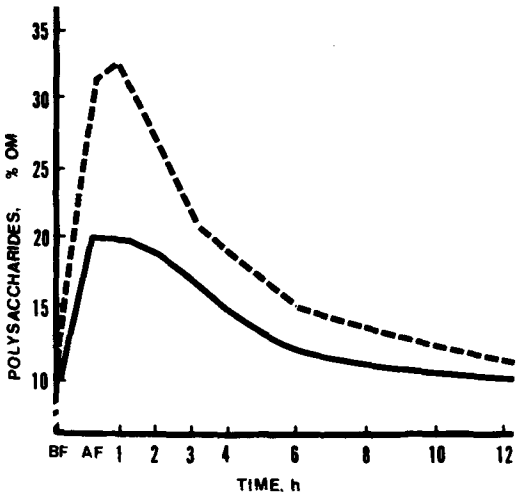

Figure 4. Change in polysaccharide concentrations in organic matter (OM) of fluid- (▬) and particle-associated (▬ ▬) microorganisms. Standard error of treatment X hour means from analysis of variance was .89%.

tions. No differences (P>.10) over time were detected in lipid concentrations of particle-associated microorganisms. Across time-points, fluid-associated microbes had higher (P<.05) lipid concentrations than particle-associated microbes. Mean lipid concentrations of fluid-associated microbes was 24.2 ± 2.6% of OM compared with 22.4 ± 1.9% for particle-associated microbes.

The changes in polysaccharide concentration (figure 4) due to feeding were opposite those of N, NA and fluid microbial lipids. Within 1 h of initiation of feeding, polysaccharide concentrations were higher (P<.01) than before-feeding concentrations in both populations, and remained significantly higher until 6 h after feed removal. Polysaccharides increased twofold from 10.4 ± 9% before feeding to 20.3 ± 1.6% 1 h after feed removal in the fluid population, and threefold from 11.8 ± .1 to 33.6 ± 1.8% at those time-points in the particle-associated population. Across time, particle-associated microorganisms contained higher (P<.01) polysaccharide concentrations than fluid microorganisms. Comparisons at individual time points showed adherent microbes were higher (P<.05) in polysaccharides than free microbes from 1 h after initiation of feeding to 8 h after feed removal.

Discussion

A typical approach to obtain a representative sample of ruminal microorganisms is to use differential centrifugation, discarding the protozoa and particulate fraction sedimenting at slow speeds and keeping the bacterial fraction subsequently pelleted at high speeds. Differential centrifugation was not used in this study because the goal was to measure total microorganisms in both populations. Protozoa and fungi that sediment at slow speeds can play an important role in ruminal function, and a large quantity of bacteria are also present in the form of clumps which sediment with protozoa and small particles (Dehority and Grubb, 1980; Olubobokun et al., 1986). A preliminary experiment in which cows consumed a diet similar to that fed in this study and in which strained ruminal fliud was filtered through eight layers of cheesecloth, indicated that the protozoal fraction (500 × g for 5 min) contained approximately twofold more DM than the bacterial fraction (30,000 × g for 30 min). The N concentration of the protozoal fraction was only slightly lower than that of the bacterial fraction (7.2 vs 7.8% of DM). Therefore, total microbial DM, N and other constituents were measured after centrifugation of strained fluid at 30,000 × g for 30 min. The ash and N concentrations observed in this study are within the range reported for ruminal bacteria (Smith, 1975; Leedle et al., 1982; Merry and McAllan, 1983; Storm and Ørskov, 1983).

The differences in composition found between fluid- and particle-associated ruminal microorganism populations partially agree with the relationships reported by others. Merry and McAllan (1983) separated liquid- and solid-associated bacteria from before-feeding ruminal contents (approximately 16 h after previous feeding). Their results were similar to our pre-feeding comparison in that fluid-associated bacteria had higher ash, higher NA, and similar carbohydrate concentrations compared with solid-associated bacteria. However, they reported significantly higher N in liquid-associated bacteria, which was detected only at 1 h after feed removal in the present study. The concentrations reported by Merry and McAllan (1983) for organic constituents of liquid- and solid-associated bacteria are comparable to those of fluid- and particle-associated microorganisms, respectively, in the current study.

Across time-points, lipid concentration of

fluid-associated microbes was higher than particle-associated microbes (24.2 vs 22.4% of OM). Merry and McAllan (1983) reported lipid concentration of 14.7% of OM for liquid-associated microbes, which was lower than that of solid-associated microbes (26.8%). Differences between the two studies could be due to factors such as sample preparation and diet consumed. Also, values from this study represent 10 time-points over a 12-h period after feeding, while values reported by Merry and McAllan (1983) represent one sampling time (approximately 16 h after feeding).

In this study, feeding caused a decrease in N and NA concentration of both populations as well as a decrease in lipid concentrations of particle-associated microorganisms. Polysaccharide concentrations increased in both populations. Other studies have shown that fluid-associated microbes have the ability to store energy in the form of polysaccharides. This storage can occur rapidly after feeding and result in decreased concentration of N and other constituents. McAllan and Smith (1977) found that when glucose was supplemented into the rumen of calves consuming fescue hay, polysaccharide concentrations increased from 5 to 23% of microbial DM within 1 h after feeding. Polysacchardide concentrations were lower at 1 h after feeding if fescue alone was fed or if the rumen was supplemented with urea plus glucose. This indicates that available energy and N are important in determining composition changes in microorganisms.

Merry and McAllan (1983) found no difference in polysaccharide concentrations in liquid- and solid-associated bacteria 16 h after feeding. In this study, particle- as well as fluid-associated microbes responded to feeding by increasing polysaccharide and decreasing N concentrations. At 1 h after feed was removed, polysaccharide levels peaked in both populations and were higher ($P<.05$) in the particle- than the fluid-associated microbes (33.6 vs. 20.3% OM). There was a trend for the particle-associated microorganisms to maintain higher polysaccharide concentrations up to 8 h after feed removal (figure 4). Quantitative estimates (Craig et al., 1987) of the samples obtained 1 h after feed removal, using ^{15}N as a marker, indicated that the particle-associated fraction was more than four times larger in microbial mass than the fluid-associated fraction (47.5 vs 10.7 mg OM/ml strained rumen fluid equivalent). Therefore, particle-associated microorganisms may play a major role in obtaining rapidly digestible nutrients from plant material. More information is needed on the role of particle-associated microorganisms in digestion of various feed sources.

Nucleic acid-to-N ratios obtained in this study are similar to what would be predicted from the DNA and RNA values of ruminal bacteria reported by Bates et al. (1985). Also, the higher values obtained in this study for fluid-associated microorganisms compared with particle-associated microorganisms, agrees with observation reported by others (Merry and McAllan, 1983; Bates et al., 1985). However, the decrease in NA/N observed soon after feeding (table 2) does not agree with expected results if the increase in microbial mass is due primarily to bacteria replication. Ribonucleic acid makes up a large part of NA, and RNA/N has been shown to increase during growth of pure cultures of ruminal bacteria (Bates et al., 1985) and non-ruminal bacteria (Dennis and Bremer, 1974). Although Bates et al. (1985) found that RNA/protein increased in mixed ruminal bacteria (from the fluid fraction) within 2 h after feeding when steers were fed a high concentrate diet, this trend was not evident when sheep were fed three diets ranging from high corn silage to high concentrate. The decrease in NA/N observed in this study may reflect a change in the microbial population, and may not be due solely to an increase in the bacterial population. For example, protozoa contain less NA than bacteria (Hungate, 1966) and protozoa have been shown to attach rapidly to ingested plant material (Orpin and Letcher, 1978).

Results of this study and those of others are interpreted to indicate that the chemical composition of both fluid- and particle-associated microorganisms varies with time after feeding. Microbial sampling must be carefully defined with respect to schedule of nutrient supply.

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